

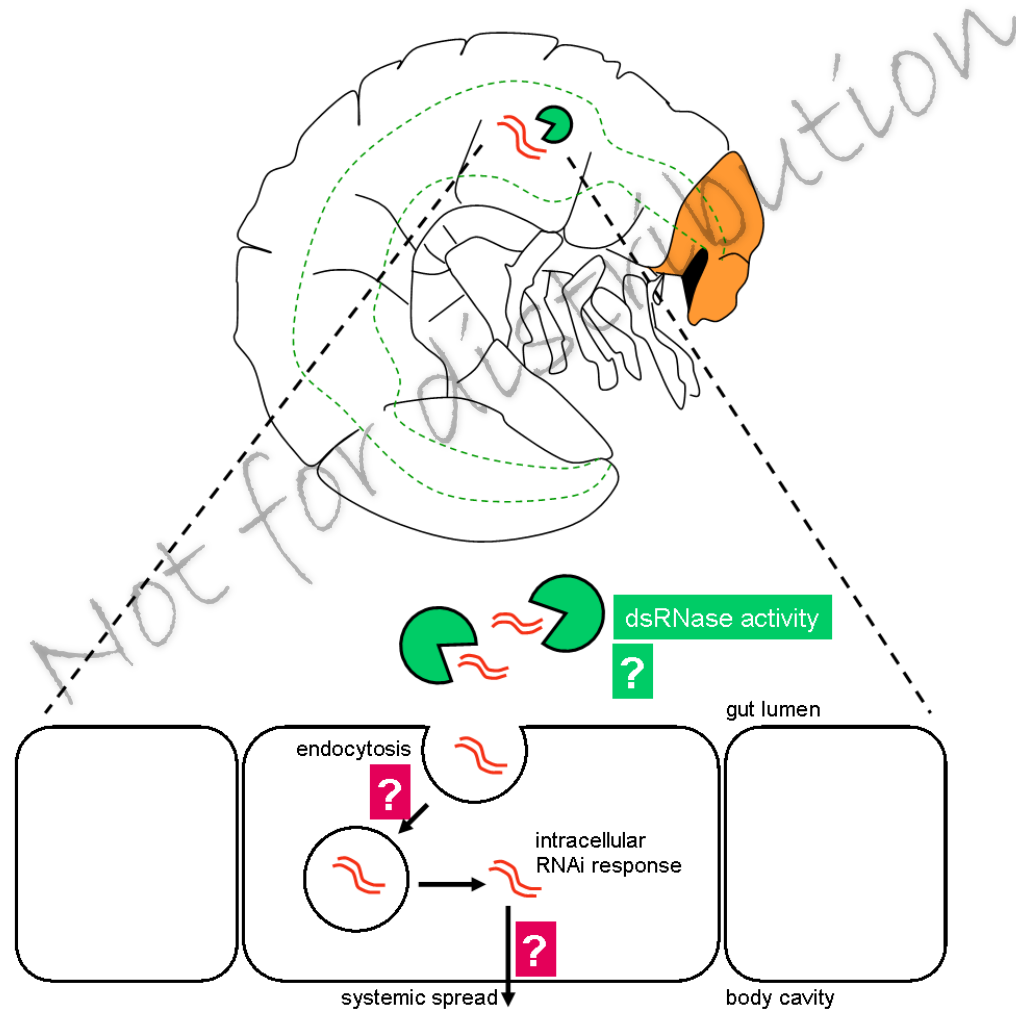
UPTAKE AND DEGRADATION OF dsRNA IN THE GUT LUMEN

in relation to environmental RNAi
efficiency

Dr. Jornt Spit



Research questions



Two insect model species

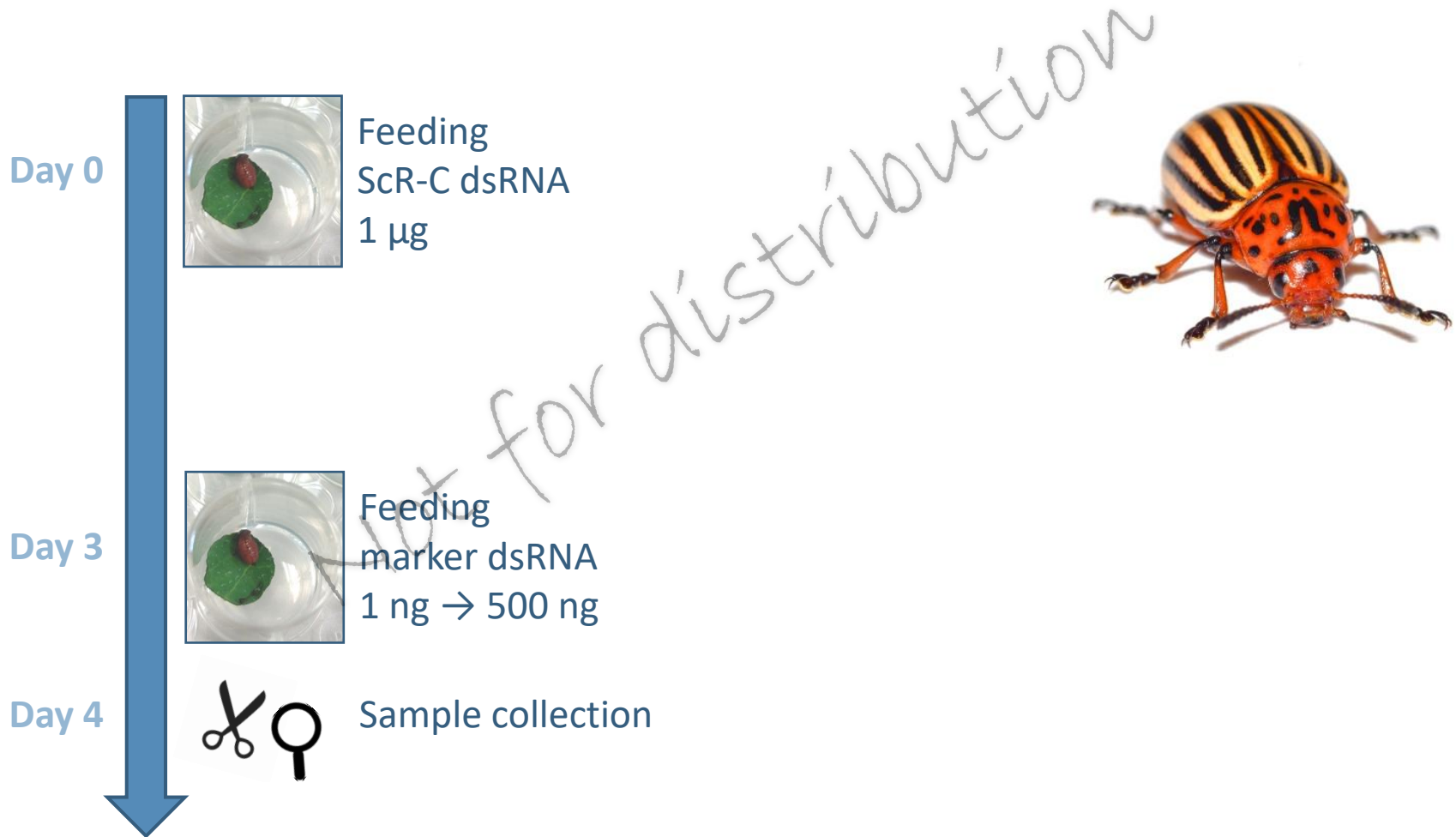


- ✓ Very potent systemic RNAi response upon injection
- ✓ No response upon feeding
- ✓ High nuclease activity

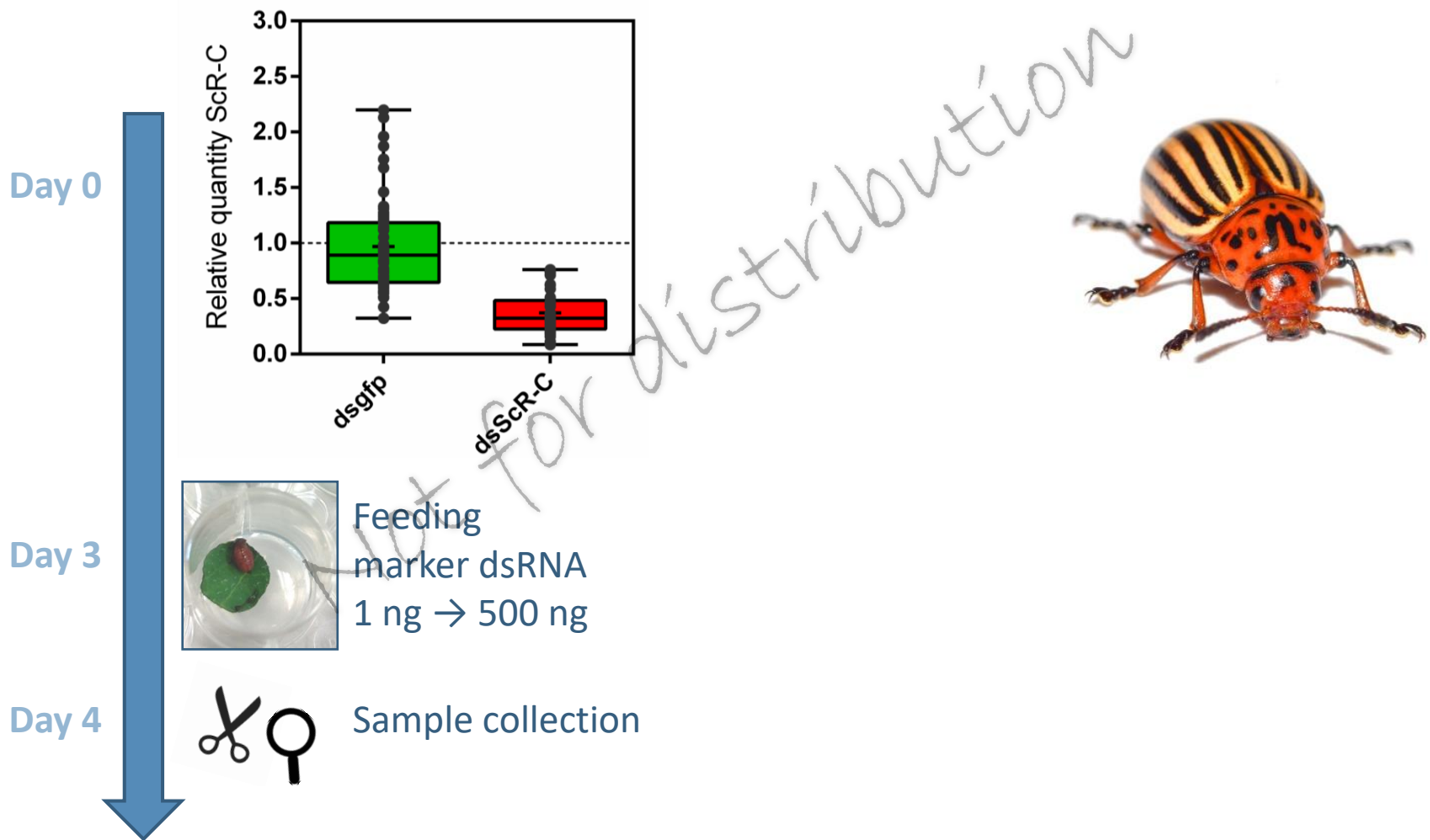


- ✓ Potent systemic RNAi response upon injection and feeding
- ✓ Low nuclease activity?

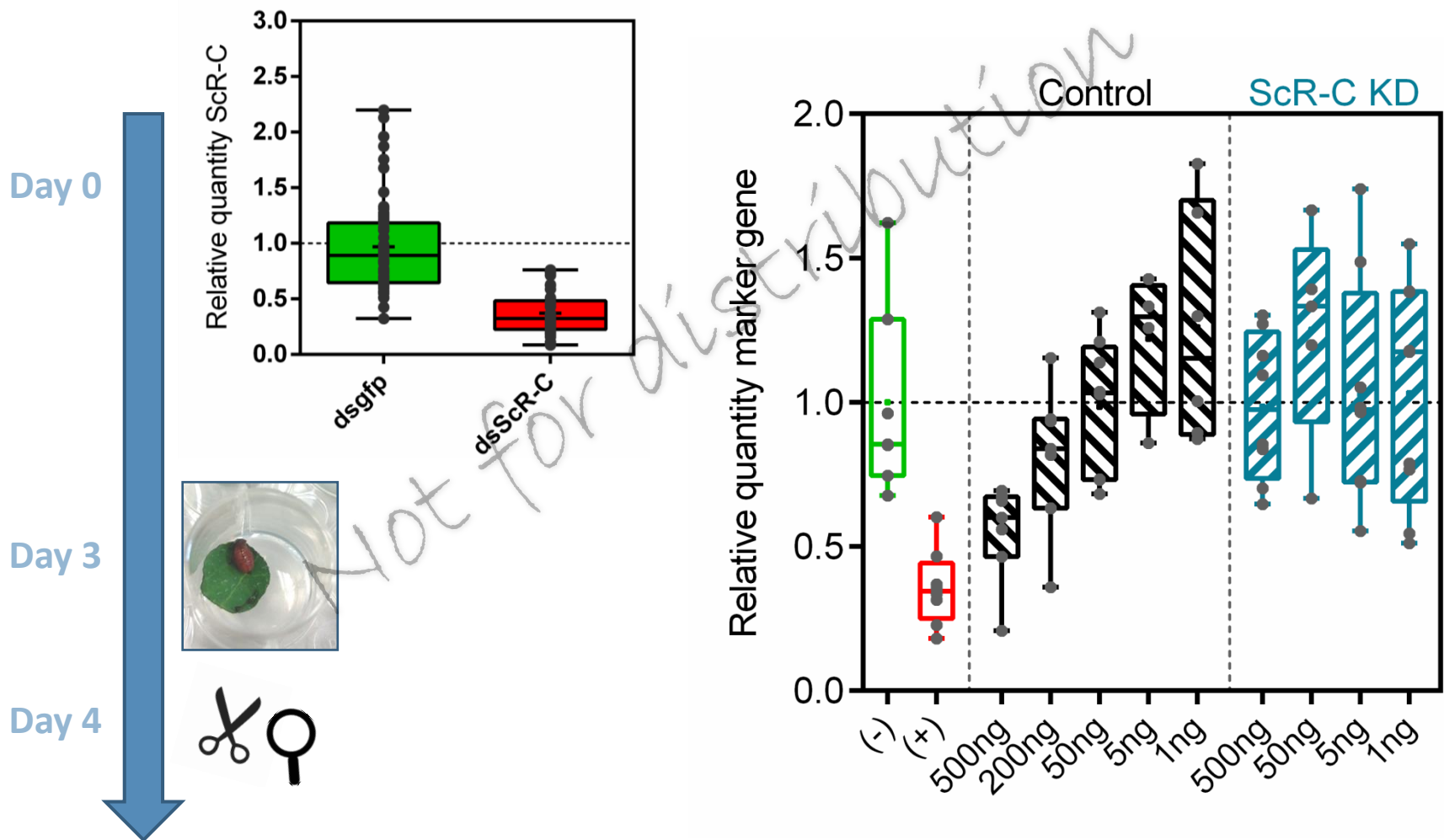
C-type Scavenger receptor involved in uptake from the gut lumen in CPB



C-type Scavenger receptor involved in uptake from the gut lumen



C-type Scavenger receptor involved in uptake from the gut lumen



Nuclease activity in the gut lumen of CPB

✓ Identification of two nuclease sequences from public sequence data

❖ *Ld_dsRNase1*

❖ *Ld_dsRNase2*

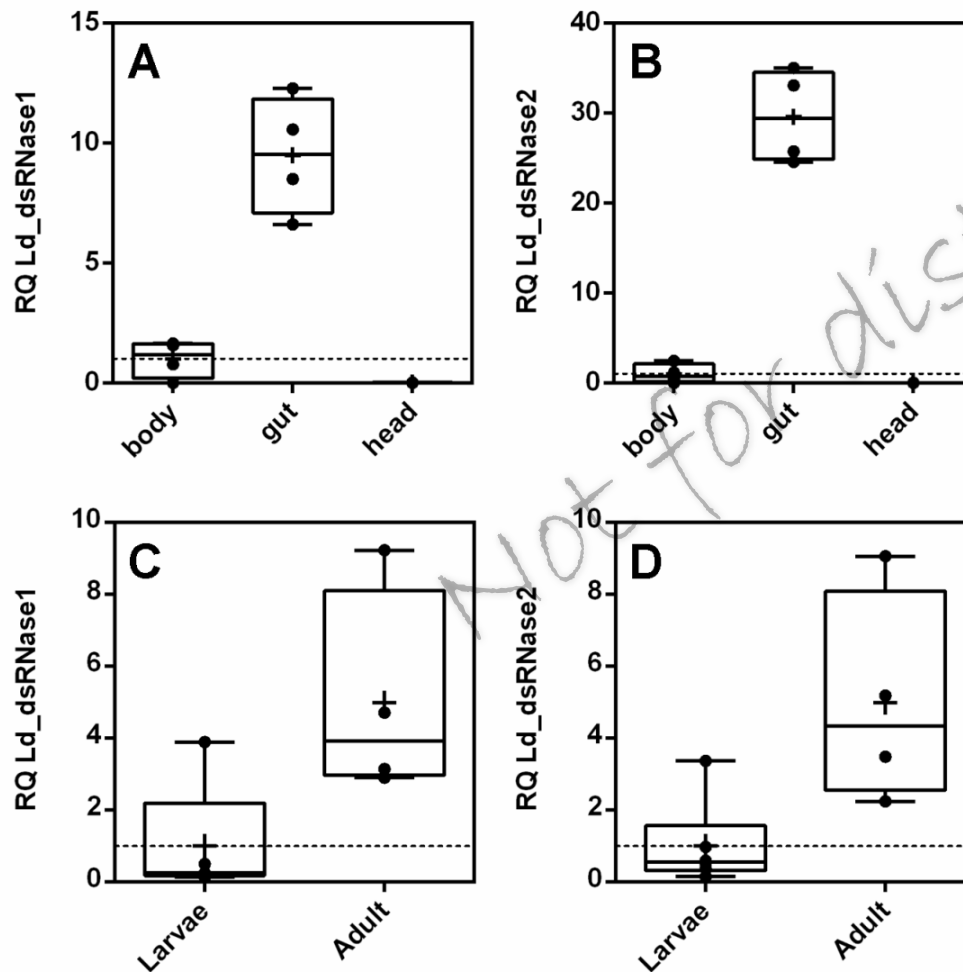


CPB transcriptome data

Assembly data set (compressed tar.gz)	Notes	SRA Genbank link	Reference	Blast xml file
454_Combined_assembly	Combined assembly of three beetle data sets (whole adult; whole larva, larval midgut), normalized library, sequencing platform: Roche 454	SRX383897 Adult	Kumar et al (2014) PLoS ONE 9(1): e86012.	Blastx
		SRX456146 Larvae	Kumar et al (2014) PLoS ONE 9(1): e86012.	
		SRX017239 midgut larvae	Pauchet et al. (2010)	

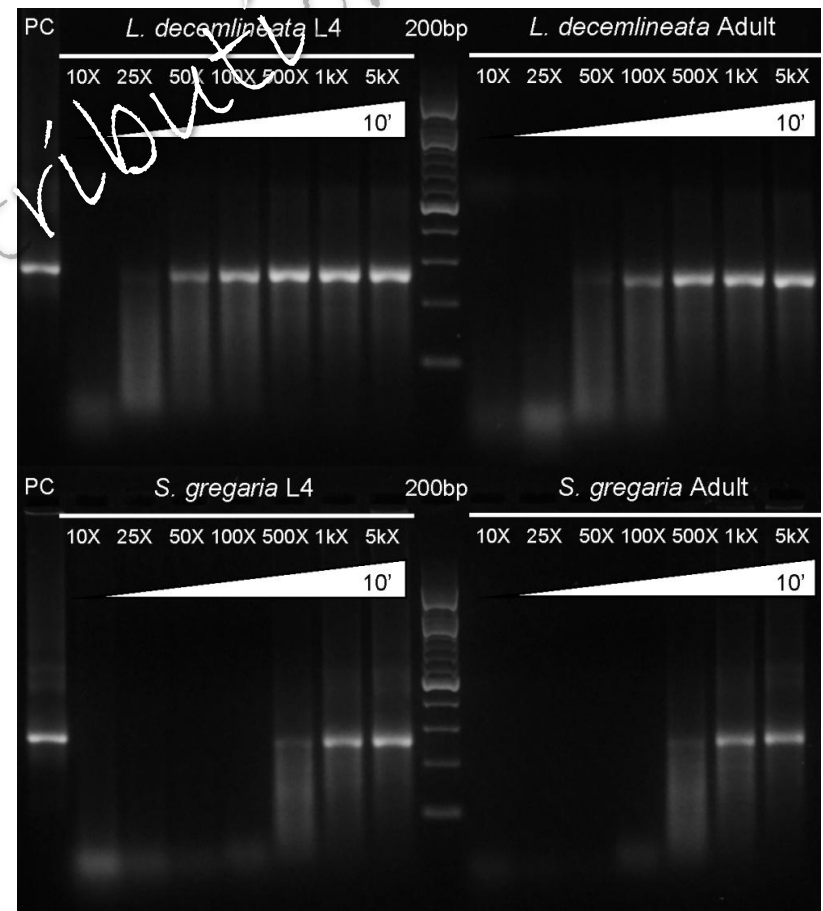
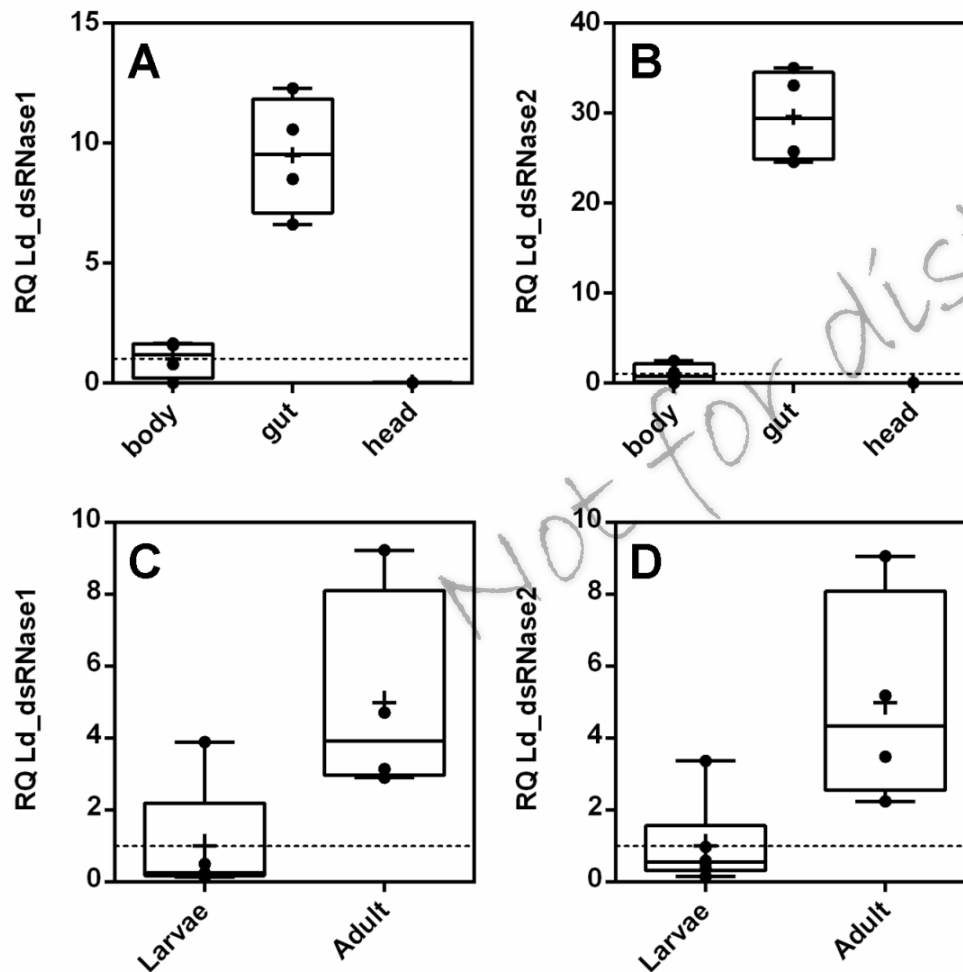
Nuclease activity in the gut lumen of CPB

✓ Identification of two nuclease sequences from public sequence data

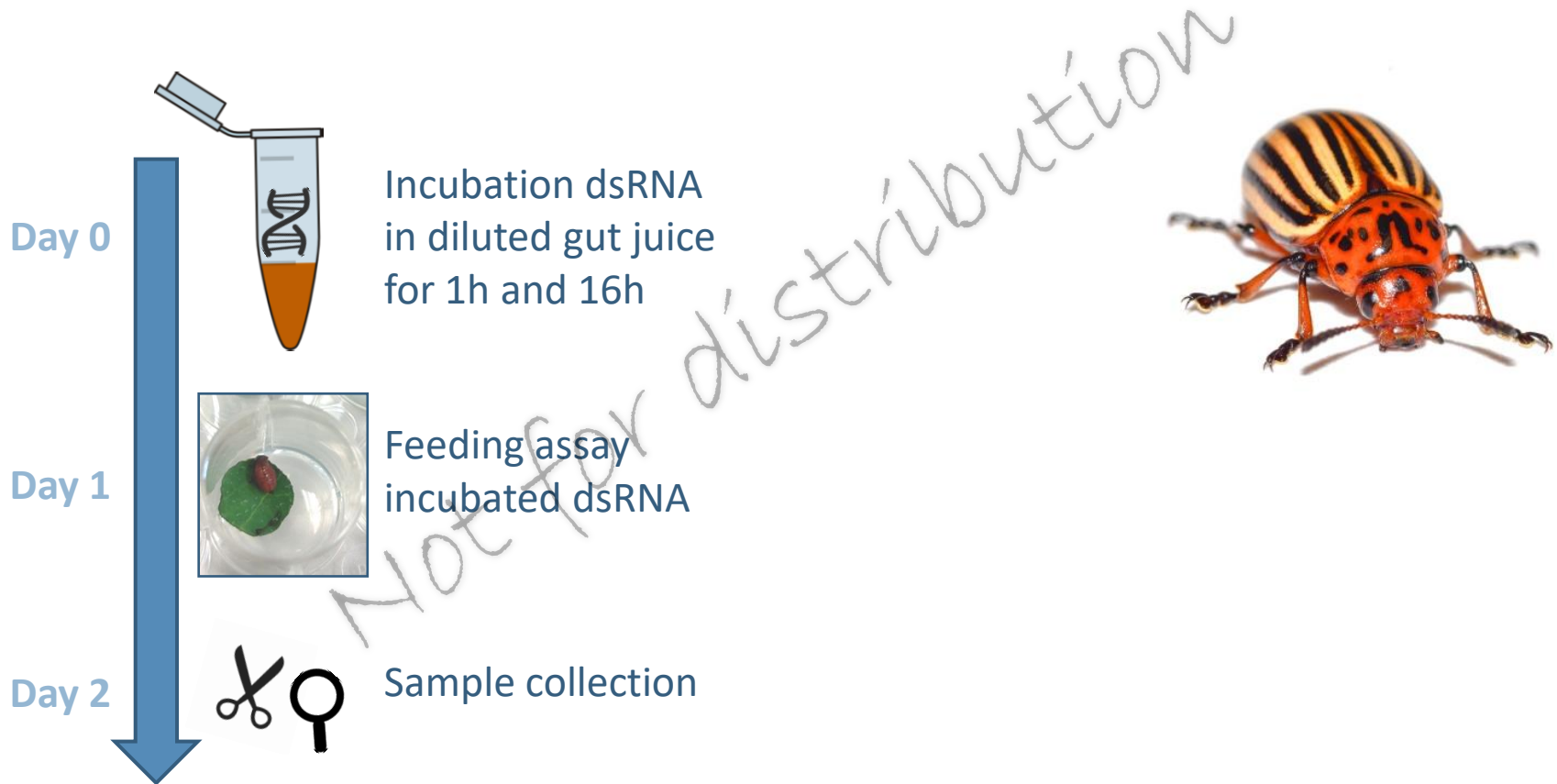


Nuclease activity in the gut lumen of CPB

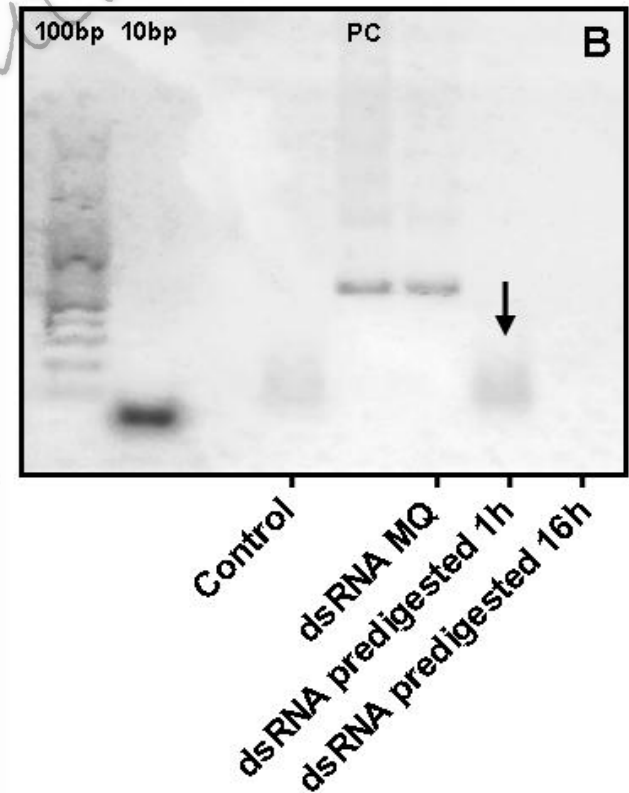
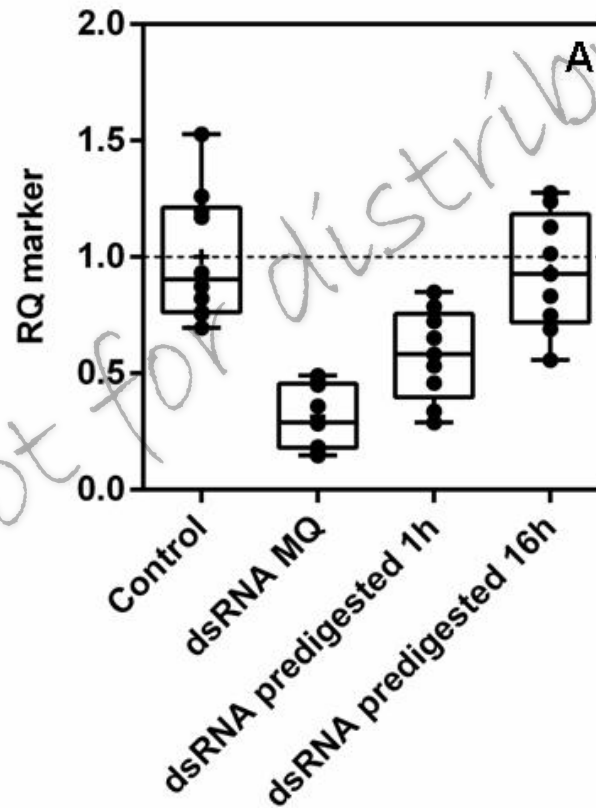
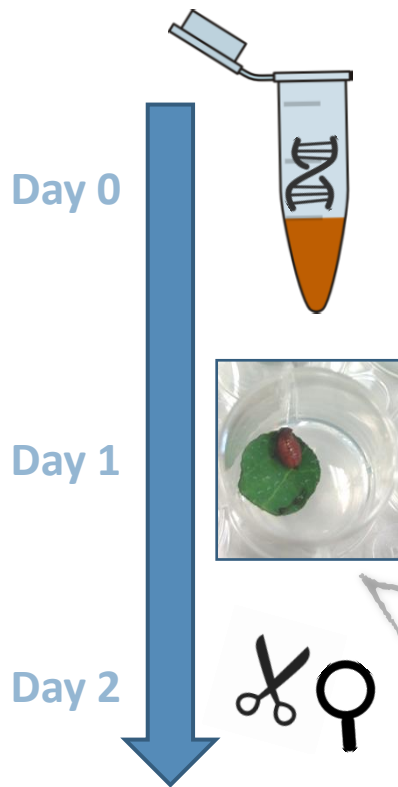
✓ Identification of two nuclease sequences from public sequence data



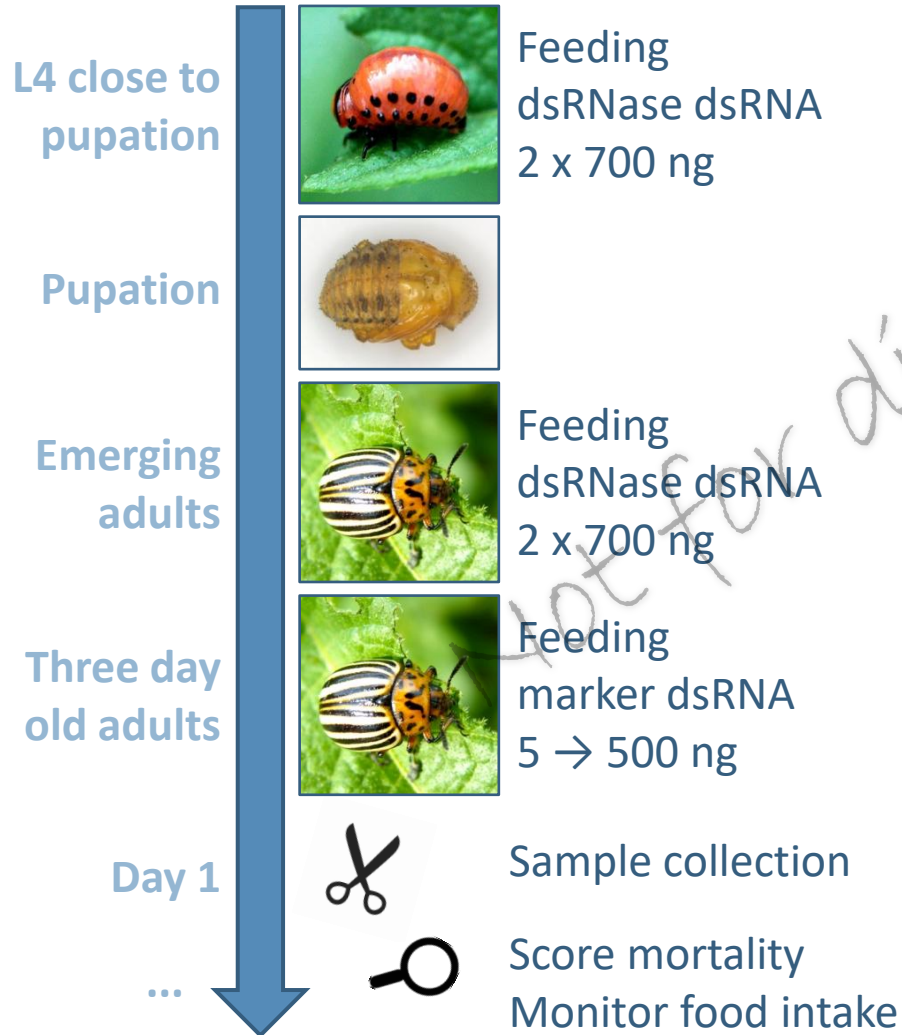
Preincubation of dsRNA simulates increased lumen nuclease activity



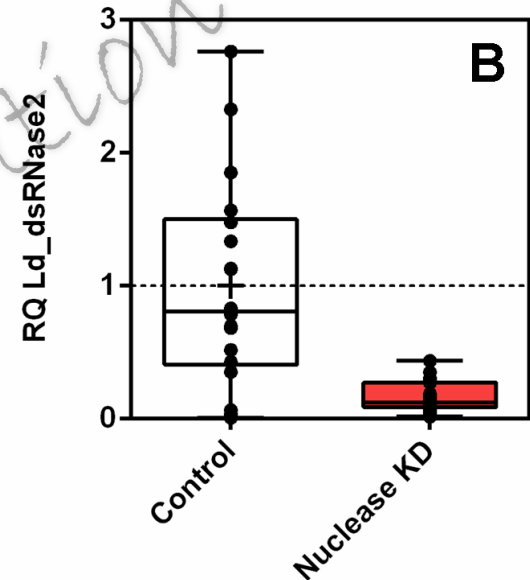
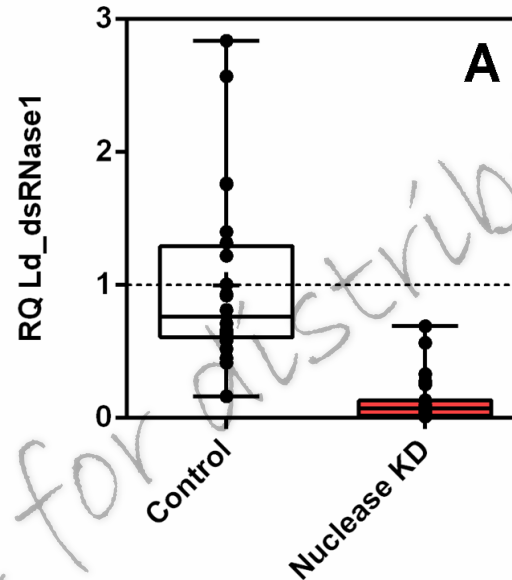
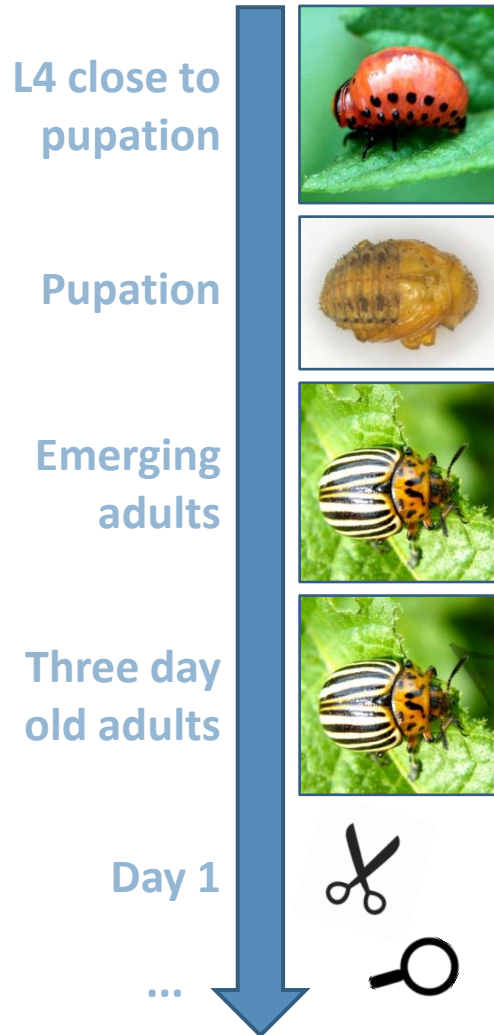
Preincubation of dsRNA simulates increased lumen nuclease activity



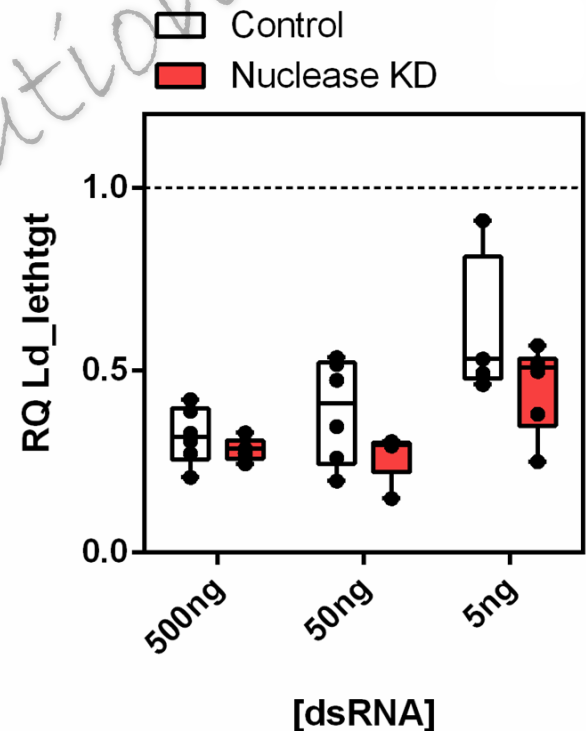
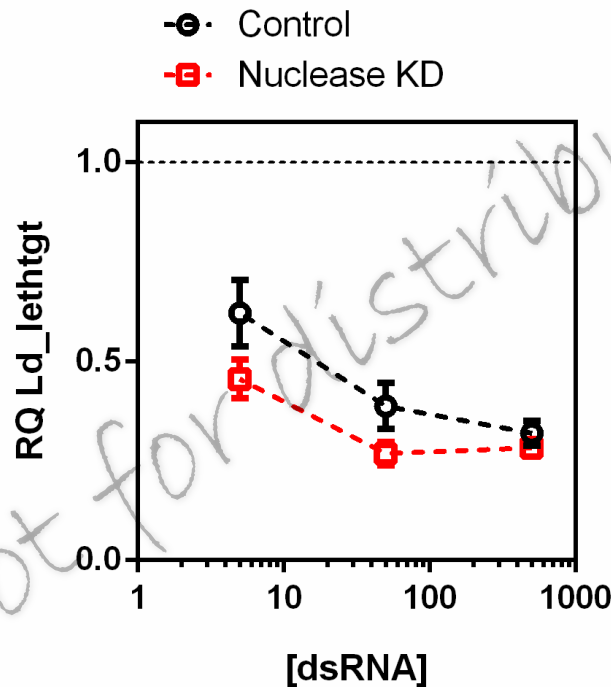
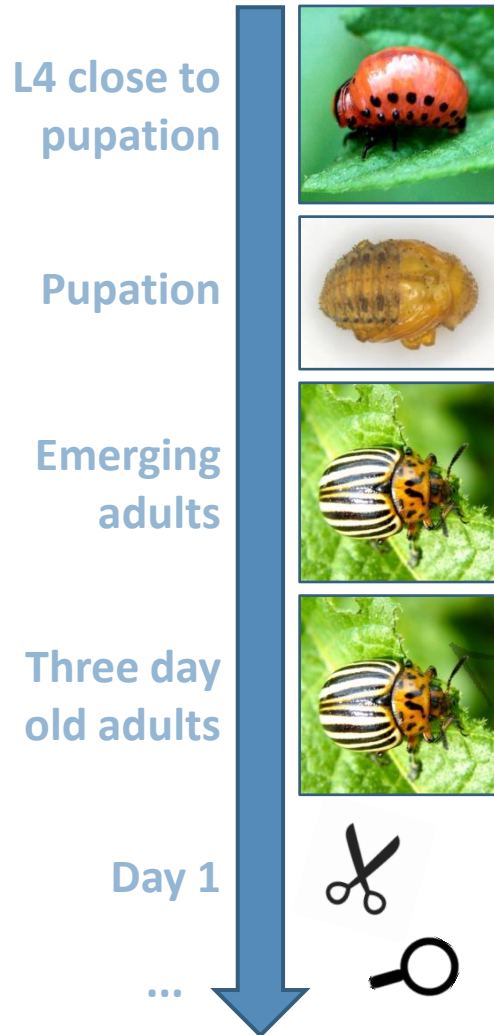
Removal of nuclease activity improves knockdown efficiency in adult CPB



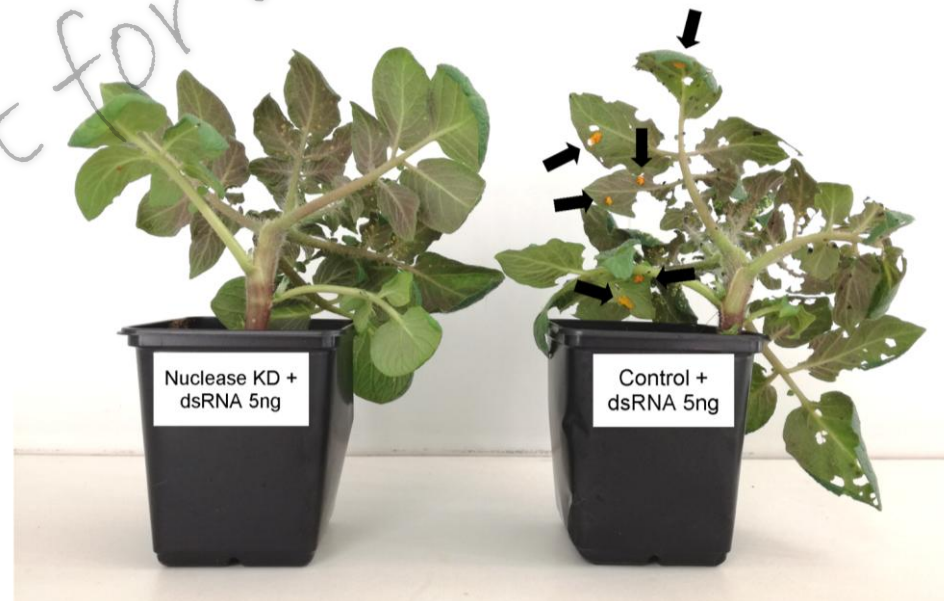
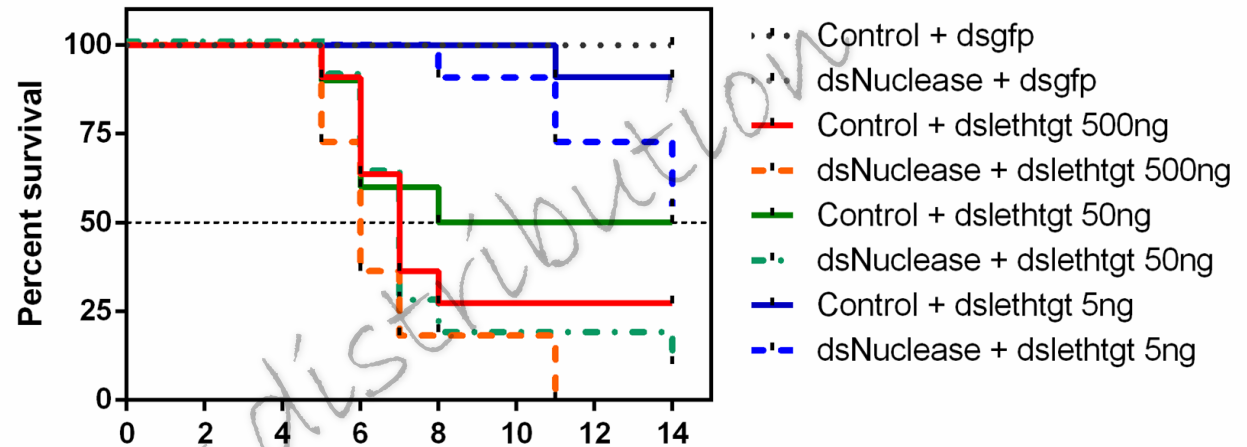
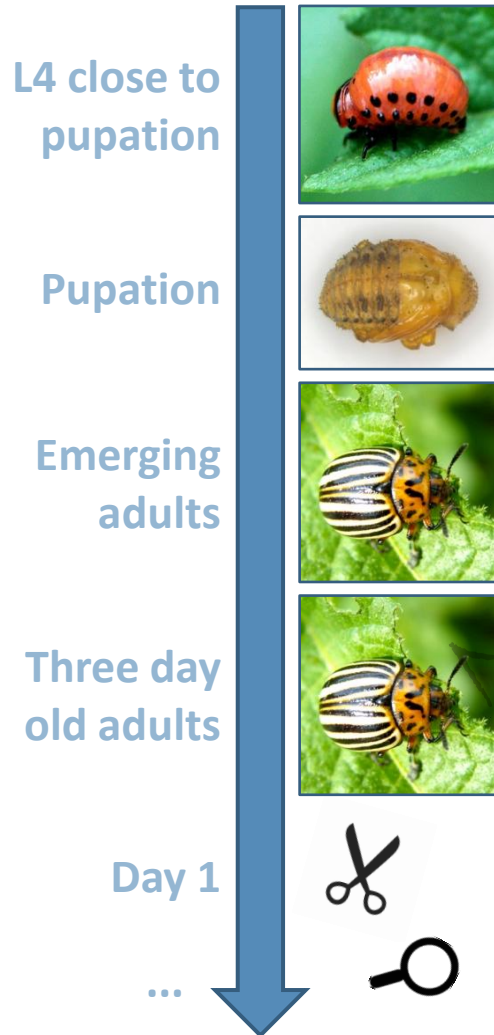
Removal of nuclease activity improves knockdown efficiency in adult CPB



Removal of nuclease activity improves knockdown efficiency in adult CPB



Removal of nuclease activity improves knockdown efficiency in adult CPB



Knockdown of nuclease activity in *Schistocerca* does not improve RNAi response



Injection adults
dsRNase dsRNA
4 x 200 ng



Day 0

Feeding
marker dsRNA
20 μ g



Day 7

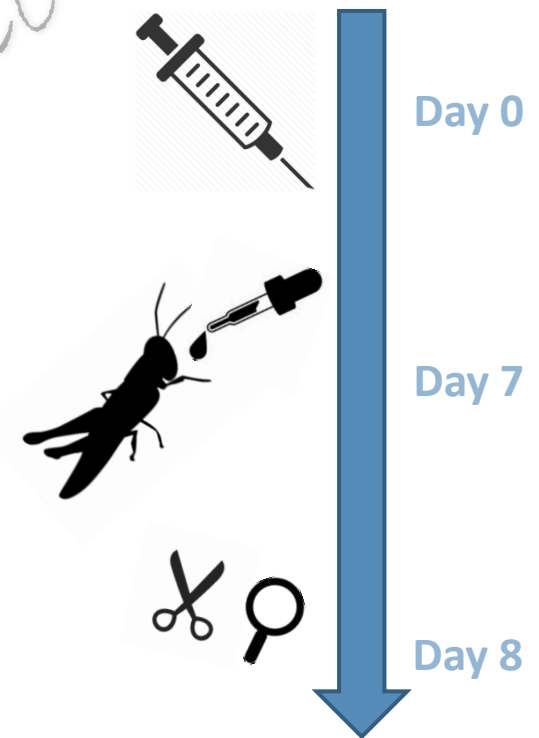
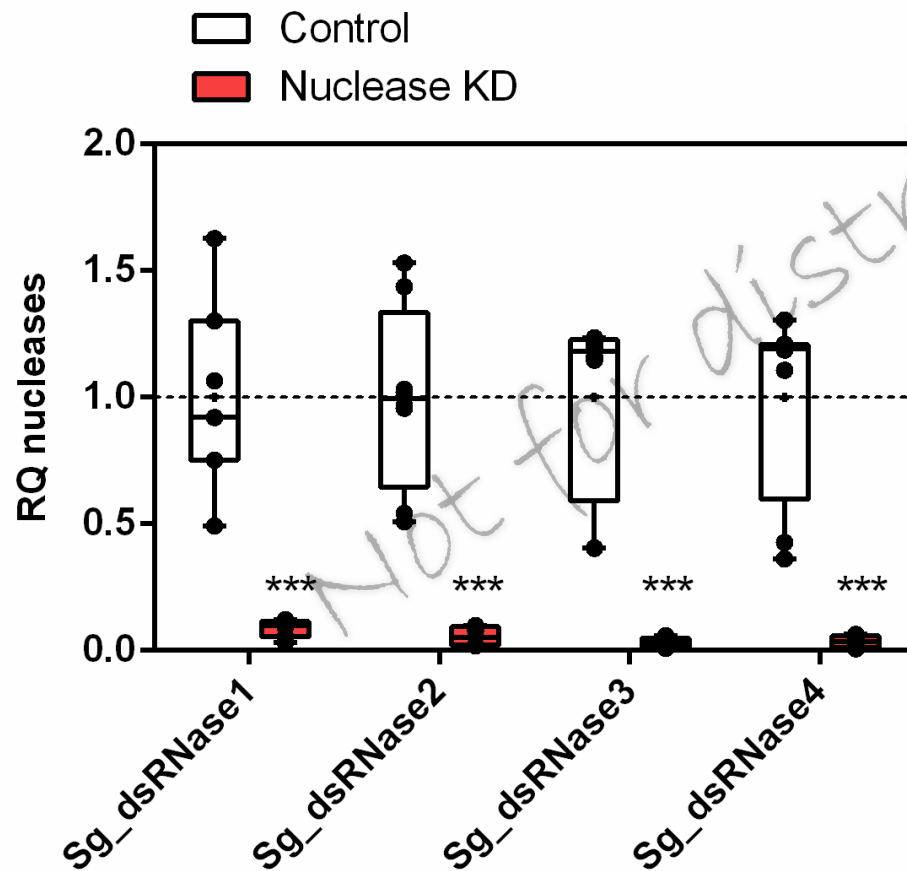
Sample collection



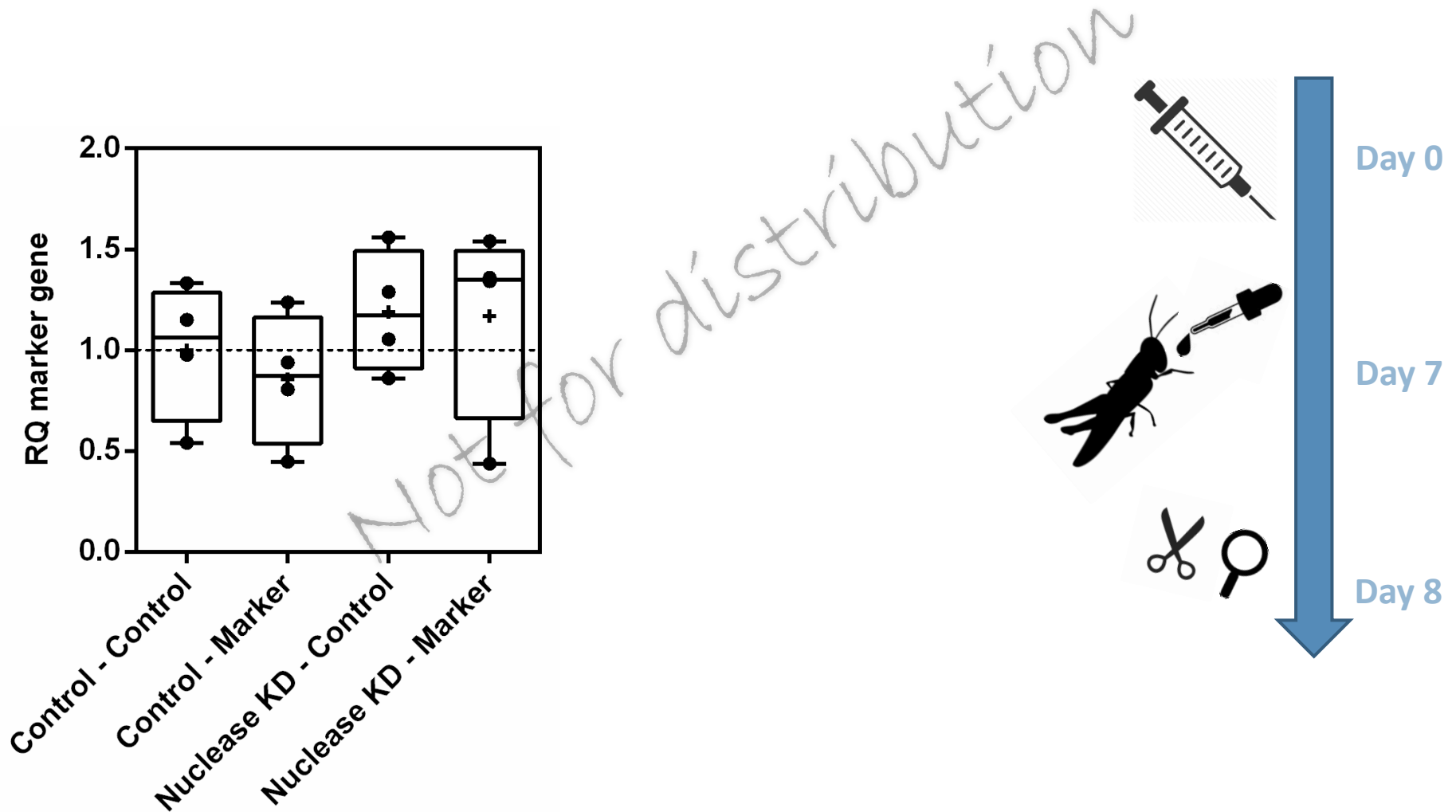
Day 8



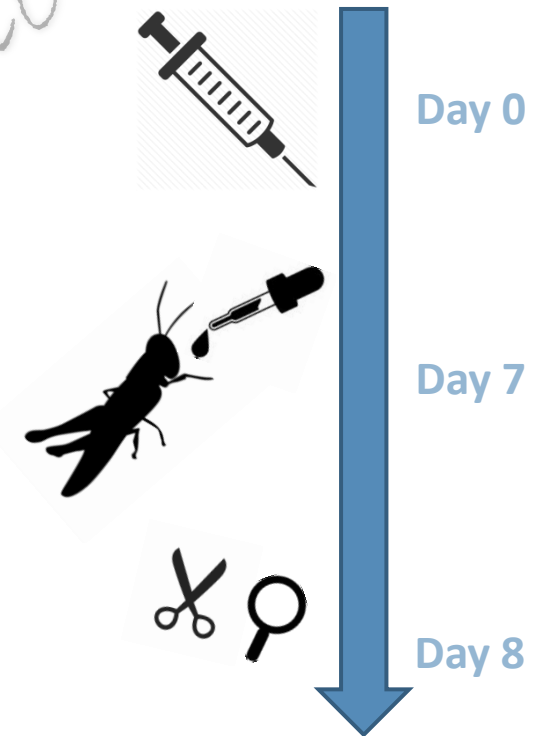
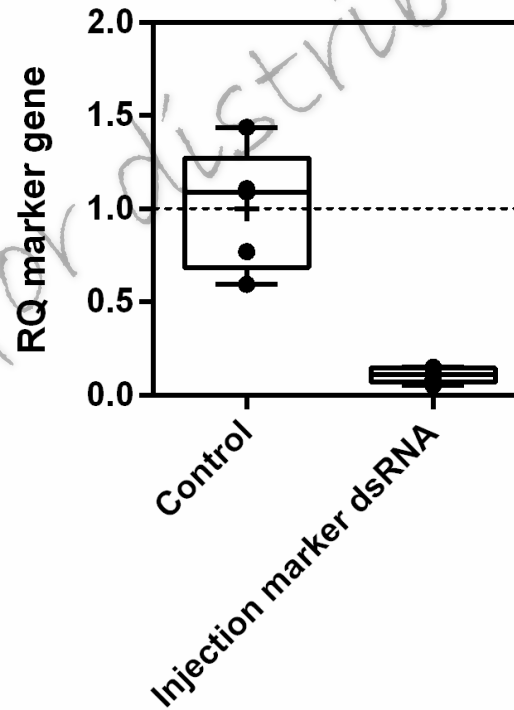
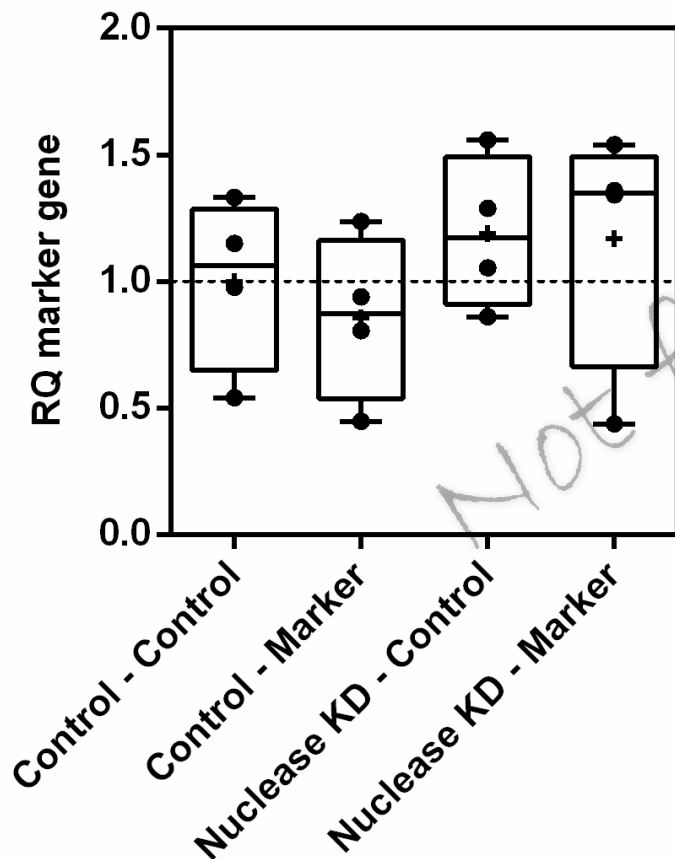
Knockdown of nuclease activity in *Schistocerca* does not improve RNAi response



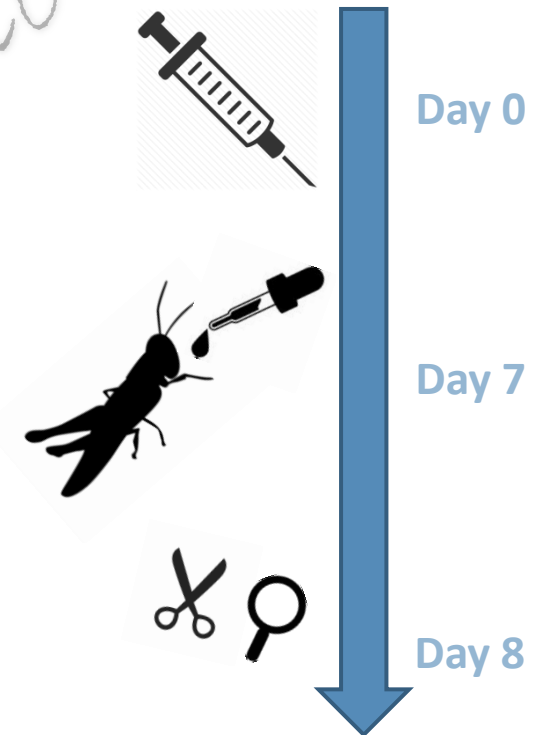
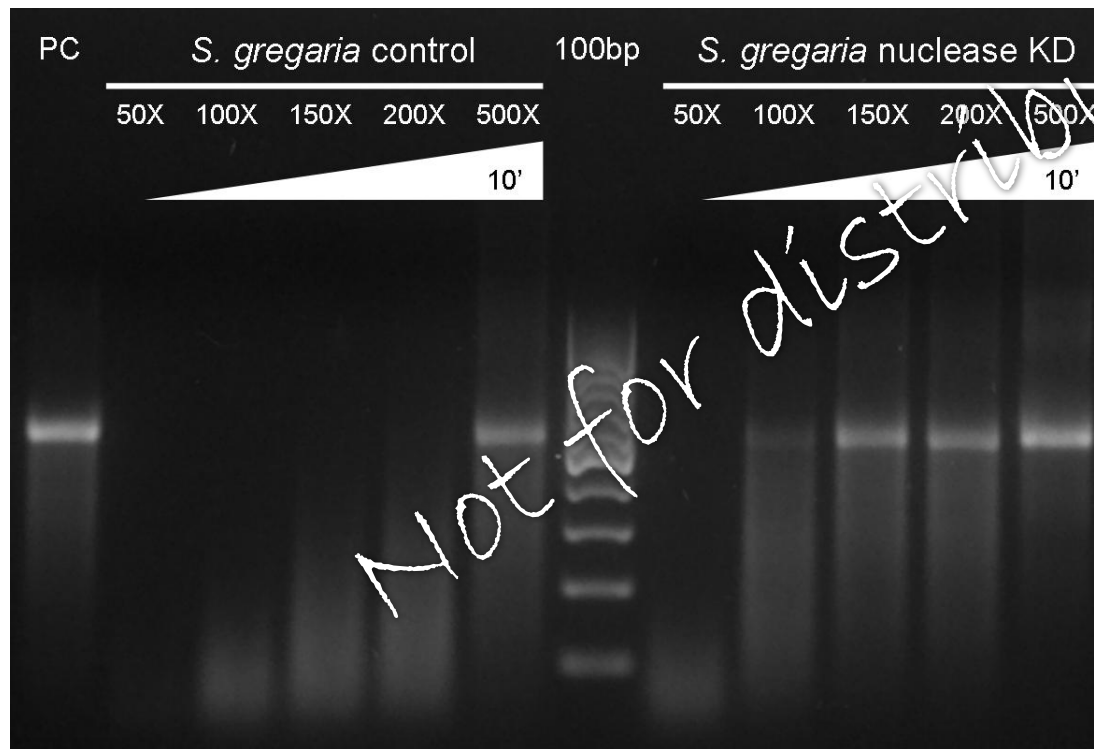
Knockdown of nuclease activity in *Schistocerca* does not improve RNAi response



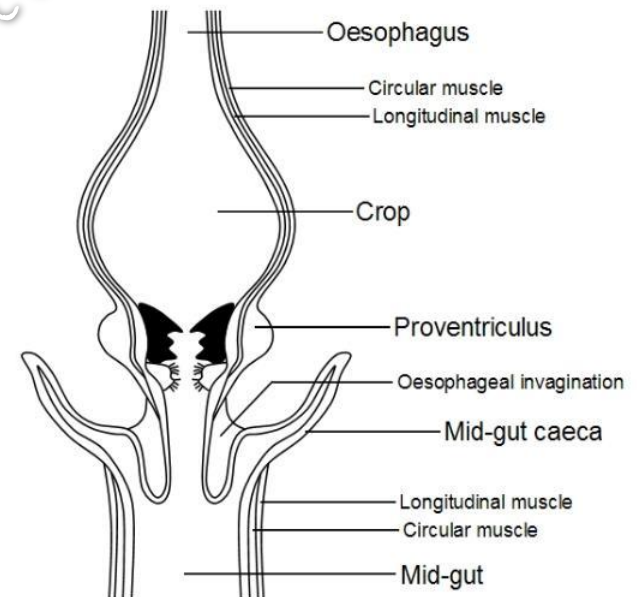
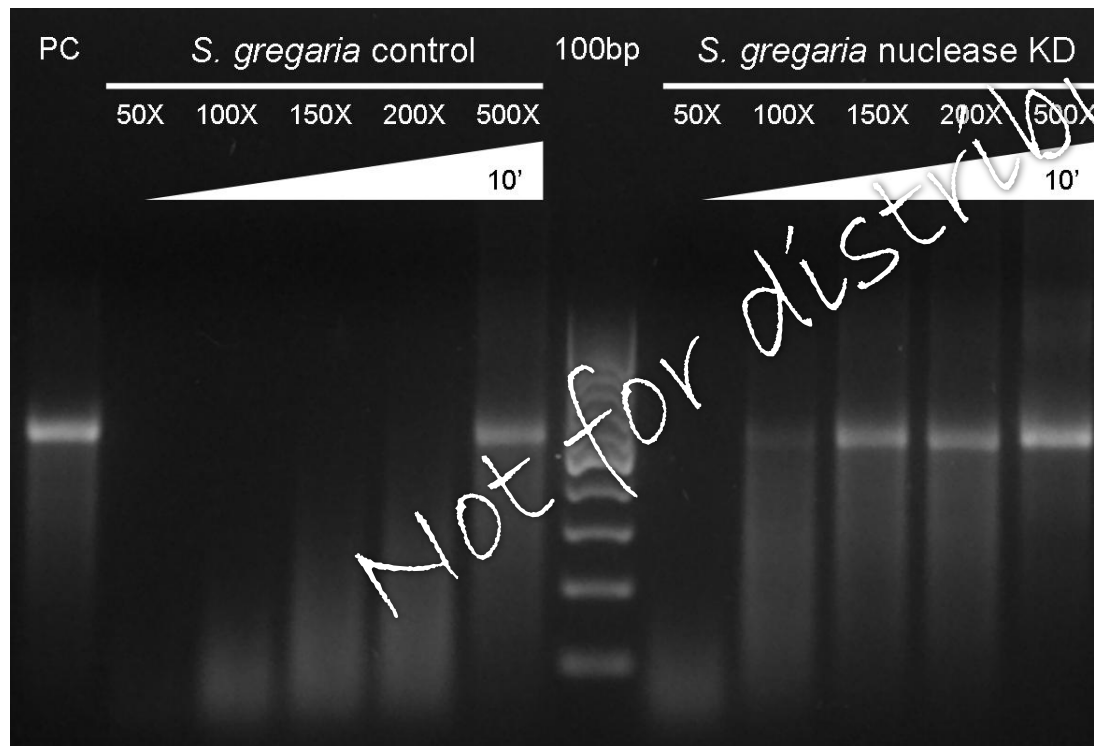
Knockdown of nuclease activity in *Schistocerca* does not improve RNAi response



Knockdown of nuclease activity in *Schistocerca* does not improve RNAi response

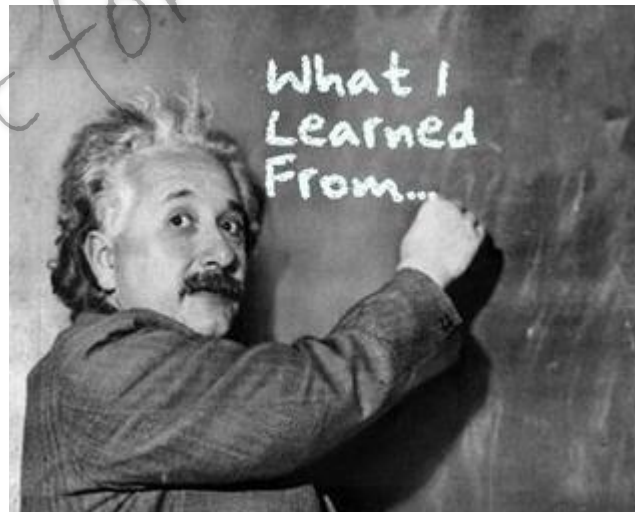


Knockdown of nuclease activity in *Schistocerca* does not improve RNAi response



Take home messages

- ✓ Nuclease genes can also be identified in Coleoptera
- ✓ Nuclease activity in the gut contributes to a lower RNAi response
- ✓ Reducing nuclease activity sensitizes the RNAi response in adult CPB, but not in the desert locust



Acknowledgements



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